templates (Kramer, F.R., and Mills, D.R., 1978). Also, 3'-O-methyl-ribonucleoside-5'-triphosphates have been used for sequencing DNA templates with *E. coli* RNA polymerase ((Axelrod, V.D., et al., 1978). None of these techniques is commonly used at present, perhaps in part, due to the difficulty to obtain the 3'-deoxy- and 3'-O-methyl-nucleoside triphosphate substrates, while 2',3'-dideoxy-ribonucleoside-5'-triphosphates that are commercially available have not been found to be substrates for wild-type (w.t.) RNA polymerases.

In view of the numerous applications involving *in vitro* nucleic acid synthesis known in the art, it is useful to consider the properties of the key nucleic acid polymerase reagents which make these procedures possible, and which, if modified in their essential properties, might improve these procedures.

One classification of nucleic acid polymerases relies on their different template specificities (RNA or DNA), substrate specificities (rNTPs or dNTPs), and mode of initiation (*de novo* or primed). These designations usually refer to the template and substrate specificities displayed *in vivo* during the fulfillment of a polymerase's biological function.

In vitro, polymerases can display novel activities, albeit with reduced efficiency and/or under non-physiological conditions. *E. coli* DNA-directed DNA polymerase I, for example, can use RNA as a template, although there is a concomitant ~100-fold increase in dNTP K_m (Ricchetti and Buc, 1993). T7 DNA-directed RNA polymerase can also use RNA as a template (Konarska and Sharp, 1989). These are not exceptional observations

because it is a general property of polymerases that they display relaxed template specificity, at least *in vitro*.

While template specificity may be relaxed, polymerase substrate specificity is normally extremely stringent. T7 DNAP, for example, displays at least 2,000-fold selectivity for dNTPs over rNTPs, even in Mn^{++} buffer which relaxes the ability of the polymerase to discriminate between dNTPs and ddNTPs (Tabor and Richardson, 1989).

It has been reported that transcripts synthesized by a T7 RNAP Y639F mutant *in vivo* yielded 1/2-1/3 of the protein per transcript compared to transcripts synthesized by the wild-type enzyme (Makarova, et al., 1995). The latter phenotype was unique to the Y639F mutant amongst a number of other active site mutants examined for *in vivo* expression, and indicated that Y639F transcripts contained a defect that led to their being inefficiently translated.

A polymerase with an altered substrate specificity would be useful in many molecular biological applications, such as creating a nucleic acid molecule comprising at least one non-canonical nucleotide.

Summary of the Invention

We disclose herein the identification of mutant polymerases, such as T7-type RNAPs, that display the ability to use dNTPs. The mutations occur in tyrosine 639 within motif B (Delarue, et al., 1990) of T7 RNAP.

We have characterized the ability of the Y639 mutants, as well as a large number of other active site mutants, to use dNTPs in both Mg^{++} and Mn^{++} buffers. Our results point to a specialized role for tyrosine 639 in T7 RNAP--and the corresponding amino acid in other polymerases--in insuring that substrates to be added to the growing nucleic acid have

the correct structure. The results reveal that both transcript and substrate structure affect the efficiency with which the transcript is extended and show that the restriction of unprimed initiation to RNA polymerases is not due to an intrinsic property of ribo- vs. deoxynucleotides, but simply to the selectivity of the polymerase active site. The present invention provides researchers with novel polymerase reagents and improved methods that expand the structural range of nucleic acids that can be enzymatically synthesized *in vitro*.

The present invention requires a polymerase with a reduced discrimination between canonical and non-canonical nucleoside triphosphates. In a preferred embodiment of the present invention, the polymerase has a reduced discrimination between rNTPs and dNTPs. In an especially preferred embodiment, the reduced discrimination is at least 10-fold compared to wild-type enzymes.

In one embodiment, the present invention is a method for synthesizing a nucleic acid molecule that comprises at least one non-canonical nucleotide. This method comprises the steps of incubating a template nucleic acid in a reaction mixture suitable for nucleic acid polymerization containing a mutant nucleic acid polymerase and the appropriate canonical and non-canonical nucleoside triphosphates which are substrates for a mutant nucleic acid polymerase and which are desired to be incorporated into the synthesized nucleic acid molecule.

In an especially preferred form of this method, the synthesized nucleic acid molecule has an altered susceptibility to a nuclease compared to a nucleic acid which could be synthesized using the corresponding non-